CHEMICAL UNDER CONSIDERATION FOR POSSIBLE LISTING VIA THE AUTHORITATIVE BODIES MECHANISM: 2-BROMOPROPANE

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2-Bromopropane may meet the criteria for listing as known to the State to cause reproductive toxicity under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code Section 25249.5 et seq.), more commonly known as Proposition 65, via the authoritative bodies mechanism. The regulatory requirements for listing by this mechanism are set forth in Title 22, California Code of Regulations §12306[1]. The regulations include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body the Office of Environmental Health Hazard Assessment (OEHHA) uses to determine whether listing under Proposition 65 is required.

The National Toxicology Program (NTP) solely as to final reports of the National Toxicology Program's Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) is one of five institutions which have been identified as authoritative bodies for identification of chemicals as causing reproductive toxicity for the purposes of Proposition 65 (§12306(1)(3)). NTP has identified 2-bromopropane as causing reproductive toxicity. OEHHA has found that this chemical appears to be "formally identified" by NTP as causing reproductive toxicity as required by §12306(d). 2-Bromopropane is the subject of a report published by the authoritative body that concludes that the chemical causes reproductive toxicity. Also, the document specifically and accurately identifies the chemical and the document meets one or more of the criteria required by §12306(d)(2).

OEHHA also finds that the criteria in regulation for "as causing reproductive toxicity" (§12306(g)) appear to have been satisfied for 2-bromopropane. In making this evaluation, OEHHA relied upon the discussion of data by the authoritative body in making its finding that the specified chemical causes reproductive toxicity. A brief discussion of the relevant reproductive and developmental toxicity studies providing evidence for the findings is presented below. Much of the discussion is taken verbatim from the NTP-CERHR (2003) report *NTP-CERHR Monograph on the Potential Human*

[1] All further references are to Title 22 of the California Code of Regulations unless otherwise indicated.

Reproductive and Developmental Effects of 2-Bromopropane. The statement in bold reflects data and conclusions that appear to satisfy the criteria for the sufficiency of evidence for reproductive toxicity (§12306(g)). The full citation for the authoritative body document is given in this report.

Chemical Under Consideration for Possible Listing as Known to the State to Cause Reproductive Toxicity

Chemical	CAS No.	Toxicological Endpoints	Chemical Use	Reference
2-Bromopropane	75-26-3	male reproductive toxicity female reproductive toxicity	Intermediate in the synthesis of pharmaceuticals, dyes, and other organic chemicals; contaminant of 1-bromopropane.	NTP-CERHR (2003)

2-Bromopropane (2-BP) (CAS No. 75-26-3).

2-BP caused numerous reproductive system effects, including reduced testes weight, reduced sperm counts, atrophy of the seminiferous tubules, decrease in the number of ovarian follicles, decrease in uterine and ovarian weights, and an increase in irregular estrous cycles.

The NTP-CERHR has concluded that there is clear evidence of adverse effects for reproductive toxicity (males and females) in laboratory animals (NTP-CERHR, 2003). NTP-CERHR states "there is evidence that human exposure to 2-BP causes reproductive toxicity in both males and females. However, the small number of exposed individuals and uncertainties in exposure levels preclude a definitive answer. Studies reviewed by the expert panel and more recent studies clearly show that exposure to 2-BP can adversely affect the reproductive system of rodents. " "[T]he NTP judges the scientific evidence of effects in laboratory animals sufficient to conclude that 2-BP may adversely affect human development and reproduction if exposures are sufficiently high." (NTP-CERHR, 2003, p. 1).

The *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of 2-Bromopropane* by the NTP-CERHR Bromopropanes Expert Panel is incorporated into NTP-CERHR (2003) as Appendix II. The Expert Panel reviewed studies of 2-BP in that report, and some of the information reported for those studies, mostly taken verbatim from NTP-CERHR (2003, Appendix II), is summarized below. In addition, studies not available to the Expert Panel were summarized in the *NTP Monograph*, and excerpts from that summary are also given below.

"Reproductive effects observed in animal studies are similar to those observed in occupationally exposed women. Major effects noted in animal inhalation studies are outlined in Table 4-12. Nine-week inhalation studies in Wistar rats demonstrated that 2-BP targets the ovary at concentrations of ≥100 ppm (≥503 mg/m^3) and disrupts estrous cycles at concentrations ≥300 ppm (≥1,509 mg/m^3) (*32, 34*) [Kamijima et al. 1997, Yu et al., 1999]. A NOAEC [No Observed Adverse Effect Concentration] was not identified, as effects occurred at the lowest exposure levels tested. The primary estrous cycle effect was continual diestrus with occasional estrus, while a smaller number of rats experienced prolonged estrus with occasional diestrus. 2-BP exposure reduced the numbers of ovarian primordial, antral, and growing follicles. In the most severely affected rats, ovaries contained follicles with few viable oocytes and thin granulose cell layers and no corpora lutea. No changes in LH or FSH levels were observed in these rats. A mechanistic study suggested that 2-BP induces ovarian toxicity through apoptotic destruction of primordial follicles and their oocytes (*34*). [Yu et al., 1999]

Summary of Reproductive Toxicity in Inhalation Studies in Female Rats (modified from CERHR (2003) Table 4-12).

Concentration in ppm (mg/m^3)	***Exposure Regimen***	***Sex/Species/ Strain/Number/ Age***	***Dose: Effect***	***Reference***
100 (503) 300 (1,509) 1,000 (5,031)	8h/9wk; whole body	Female Wistar Rat 7-9/group age 15 weeks	**100 ppm (503mg/m^3):** ↓Primordial and growing follicles **300 ppm (1,500 mg/m^3):** Disrupted estrous cycle; ↓ Primordial, growing, and antral follicles ↓ Absolute and relative uterus weight **1000 ppm (5,031 mg/m^3):** Disrupted estrus cycle ↓ Absolute ovary weight and absolute and relative uterus weight ↓ Primordial, growing, and antral follicles ↑ Atretic and cystic follicles ↓ Viable oocytes, and no corporal lutea	Kamijima et al. 1997 Yu et al. 1999

"Reproductive effects in male rats exposed to 2-BP were reported in two inhalation studies; major effects in these studies are outlined in Table 4-13. Inhalation exposure to ≥300 ppm (≥1,509 mg/m^3) for at least 9 weeks resulted in atrophy of seminiferous tubules, reductions in germ cell numbers, and hyperplastic Leydig cells (*22, 26*) [Ichihara et al., 1997; Yu et al., 2001] in addition to reduced sperm counts and motility with increased numbers of abnormal sperm (*22*) [Ichihara et al., 1997]. Although

inhalation is the primary route of human exposure, testicular lesions, sperm effects, and or Leydig cell hyperplasia were also observed in animal studies with ip, sc, or oral exposure (*25, 37, 43*) [Yu et al., 1997; Wu et al., 1999; Son et al., 1999]. Although limited, a sc injection study in male rats treated with ≥600 mg/kg bw 2-BP for 5 weeks demonstrated reductions in mating and fertility (37) [Wu et al., 1999].

"A series of studies demonstrated that acute, high-dose, parenteral exposure to 2-BP causes apoptotic death of spermatogonia within 3 days of exposure (*38, 40, 43, 45*) [Omura et al., 1997, 1999; Son et al., 1999; Yu et al., 2001]. 2-BP exposure also resulted in apoptosis in spermatocytes at about 9 days after the end of a 5-day treatment (*45*) [Yu et al., 2001]."

Summary of Reproductive Toxicity in Inhalation Studies in Males Rats (modified from CERHR-NTP (2003) Table 4-13)

Concentration in ppm (mg/ m^3)	*Exposure Regimen*	*Sex/ Species/ Strain/ Number/ Age*	*Dose: Effect*[a]	*Reference*
300 (1,509) 1,000 (5,031) 3,000 (15,092)	8h/7d/9wk; whole body (9-11 d exposure period in high dose)	Male Wistar Rat 9/group age 13 weeks	**300 ppm (1,509 mg/m^3):** ↓ Absolute and relative epididymides and testes weight ↓ Absolute prostate and seminal vesicles weight ↓ Sperm count and motility and ↑ Abnormal sperm ↑ Seminiferous tubule atrophy and hyperplastic Leydig cells ↓ Germ cells **1,000 (5,031 mg/m^3):** ↓ Absolute and relative epididymides and testes weight ↓ Absolute prostate and seminal vesicles weight ↓ Sperm count and motility, very few intact sperm remaining ↑ Seminiferous tubule atrophy and hyperplastic Leydig cells, ↓ Germ cells. **3,000 (15,092 mg/m^3):** ↓ Absolute and relative epididymides and testes weight ↓ Absolute prostate and seminal vesicles weight ↓ Sperm count and motility ↑ Seminiferous tubule atrophy, hyperplastic Leydig cells and vacuolation of Leydig cells ↓ Germ cells	Ichihara et at., 1997
100 (503) 1,000 (5,031)	8h/7d/12wk; whole body	Male Wistar Rat 9/group Age 10 weeks	**Reproductive NOAEC=100 ppm (503 mg/m^3)** **1,000 ppm (5,031 mg/m^3) :** Seminiferous tubule atrophy ↓ germ cells.	Yu et al., 2001

[a] Non-reproductive effects for male rats are summarized in Section 4
↑=Increased Effect; ↓=Decreased Effect
h=hour, d=days; wk=week

Some studies not available to the Expert Panel summarized in the *NTP Monograph* are discussed by the NTP CERHR as follows:

"An *in vitro* study evaluated the effects of 2-BP exposure on cultured rat Leydig cells (Wu et al., 2002). The study showed that DNA damage increased as cells were exposed to increasing concentrations of 2-BP. This study also showed that 2-BP exposure increased levels of malondialdehyde and glutathione peroxidase while decreasing superoxide dismutase acitivity. The authors suggested that 2-BP induces DNA damage, impairs cellular defenses against oxidative damage, and enhances lipid peroxidation in Leydig cells. They proposed that these effects may be responsible for animal and human testicular toxicity. This study provides additional data on the possible mechanisms by which 2-BP induces male reproductive toxicity.

"Li et al. (2001) exposed mature male rats to 2-BP by subcutaneous injections of 135, 405 or 1355 mg/kg bw/day for up to 28 days. Testes were examined by histopathologic examination and electron microscopy. In animals sacrificed on days 14 and 28, treatment with 405 or 1355 mg/kg bw/day resulted in a variety of adverse effects on the testes including atrophy of the seminiferous tubules and degeneration of spermatogonia, spermatocytes and spermatids. The authors suggested that 2-BP exposure results in apoptotic death of male germ cells. Use of the subcutaneous route of exposure limits the usefulness of these data in assessing possible human health effects.

"Sekiguchi et al. (2000) exposed female rats to 500 or 1000 mg/kg bw/day 2-BP by intraperitoneal injection at 2 to 3 day intervals for 17 days. Treated animals had longer estrous cycles and degeneration of late stage ovarian follicles. Use of the intraperitoneal injection route of exposure limits the usefulness of these data in assessing possible human health effects."

"These studies provide further evidence that 2-BP exposure adversely affects the reproductive systems of female and male rodents."

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